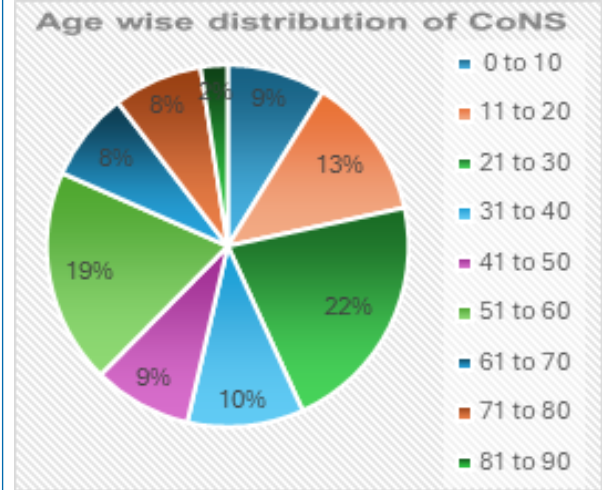


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| Dr. Arun Kumar                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Resident, Department of Microbiology, Dr. Panjabrao alias Bhausaheb Deshmukh Memorial Medical College, Amravati.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                           |
| ABSTRACT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <b>Objectives:</b> This study aimed to identify the species distribution of coagulase-negative staphylococci (CoNS) isolated from various clinical samples and to determine their antibiotic susceptibility patterns. <b>Materials And Methods:</b> A hospital based cross-sectional study was conducted from July to December 2024, involving 125 non-repetitive CoNS isolates from 1125 clinically relevant samples. CoNS were identified using standard microbiological procedures, including various biochemical tests. Antibiotic Susceptibility testing was performed by Kirby Bauer disk diffusion method, following CLSI guidelines. Vancomycin minimum inhibitory concentration (MIC) was determined using E-strips. Methicillin resistance was detected using cefoxitin disks. <b>Statistical Analysis-</b> The collected data was entered and analysed using MS-Excel version 11. <b>Result-</b> Staphylococcus hominis (42.4%) was the most prevalent species, followed by Staphylococcus schleiferi (36.8%), Staphylococcus lugdunensis (15.2%), Staphylococcus epidermidis (4.8%) and Staphylococcus haemolyticus (0.8%). The highest resistance was observed for penicillin (92%) and erythromycin (80.8%). All isolates were sensitive to vancomycin and linezolid. Methicillin resistance was detected in (42.4 %) of the isolates. <b>Conclusion:</b> Staphylococcus hominis was the most predominant CoNS species isolated. The high resistance to commonly used antibiotics like penicillin and erythromycin, along with a significant rate of methicillin resistance, highlights the need for rapid identification, speciation, and antibiotic susceptibility testing of CoNS for effective patient management in hospital settings.                                                                                                                     |                                                                                                                                                                                                           |
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| INTRODUCTION                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | susceptibility pattern.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                           |
| All staphylococcal species other than <i>Staphylococcus aureus</i> fall under the classification of Coagulase-negative Staphylococci (CoNS). Coagulase negative staphylococci were typically regarded as contaminants lacking significant clinical importance. However, these organisms are recognized as important agents of human diseases. <sup>[1]</sup> CoNS as opportunists are responsible for several health care related infections. CoNS are capable of colonizing and infecting humans through different mechanisms, such as adherence, invasion, persistence and evasion of innate and adaptive immunity. <sup>[2]</sup> The most common human pathogens include <i>S. epidermidis</i> , <i>S. hominis</i> , <i>S. haemolyticus</i> and <i>S. saprophyticus</i> . Other significant opportunistic but rarely isolated species of CoNS includes <i>S. lugdunensis</i> , <i>S. warneri</i> , <i>S. capitis</i> , <i>S. cohnii</i> , <i>S. simulans</i> , <i>S. saccharolyticus</i> , and <i>S. xylosu</i> . <sup>[3]</sup> These species have been documented as a cause of healthcare associated bacteremia, urinary tract infections, wound infections and pediatric and neonatal infections. These infections are challenging to treat due to associated risk factors and organisms' resistance to multiple drugs. <sup>[4]</sup> The increasing prevalence of methicillin-resistant coagulase-negative staphylococci (MRCoNS) in hospitalized patients is a major concern. <sup>[5]</sup> Methicillin-resistant coagulase-negative staphylococci (MRCoNS) show higher resistance to all antibiotics, specially, non-beta-lactam antimicrobials, compared to methicillin-susceptible CoNS (MSCoNS). <sup>[6]</sup> | <b>MATERIALS AND METHODS</b><br>A hospital-based cross-sectional study was undertaken in the Department of Microbiology at tertiary care hospital. The study was conducted after obtaining the approval from the Institutional ethics Committee. The study was carried out from July 2024 to December 2024. This study included 125 consecutive, non-repetitive CoNS isolates obtained from 1125 clinically relevant samples (both inpatients and outpatients) received in the Department of Microbiology during the study period. Informed consent was obtained from the patients prior to inclusion. Patients with a history of prior antibiotic administration, those unwilling to participate and samples exhibiting polymicrobial growth were excluded.<br><br>Clinical samples including pus, blood, urine, wound swab, catheter tip, ascitic fluid, sputum were processed and CoNS were identified using standard operating procedure. <sup>[7]</sup> These samples were inoculated on to nutrient agar, blood agar and MacConkey agar and incubated aerobically at 37°C for 18-24 hours. Blood samples were sub-cultured on 2 <sup>nd</sup> and 7 <sup>th</sup> day of incubation. Initial identification of CoNS was based on colony morphology, gram staining, catalase test, slide coagulase test and tube coagulase test. Standard procedures including ornithine decarboxylase and urease activity test, as well as susceptibility testing to Novobiocin and Polymyxin B (50 unit), were used for CoNS speciation. <sup>[8]</sup> Antibiotic Susceptibility testing was conducted by Kirby-Bauer disk diffusion method adhering to Clinical and Laboratory Standards Institute (CLSI) guidelines. <sup>[9]</sup> Commercially available disks of penicillin (10 U), erythromycin (15 ug), Clindamycin (2 ug), Gentamycin (10 ug), Cotrimoxazole (1.25/23.75 ug), |                                                                                                                                                                                                           |
| Studies on prevalence of CoNS helps in understanding their distribution and resistance to antibiotics. It will be very useful to develop new therapeutic strategies to prevent and treat CoNS infections. Thus, the present study was carried out to identify the various species of coagulase-negative staphylococci (CoNS) and to determine their antibiotic                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                           |
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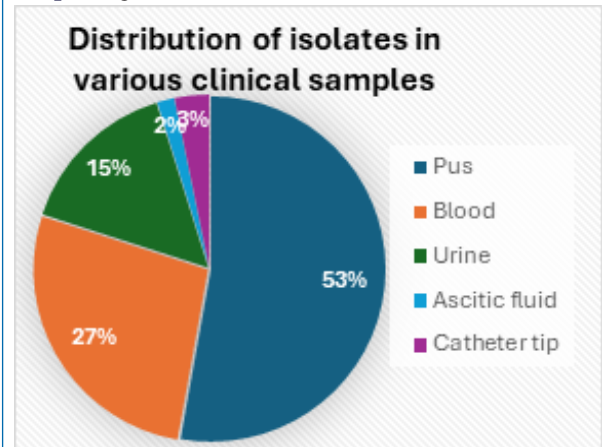
Ciprofloxacin (5 ug), Linezolid (30 ug) and Nitrofurantoin (300 ug) (for urinary isolates) were utilized. Vancomycin minimum inhibitory concentration (MIC) was determined using E-strips from HIMEDIA. Quality control for this testing involved ATCC strain, *Staphylococcus aureus* 25923. Methicillin resistance in CoNS isolates was detected using a 30 ug cefoxitin disks by disk diffusion method. Isolates with a zone of inhibition greater than 25 mm were reported as sensitive and those with a zone less than 24 mm as resistant, with exception of *S. lugdunensis*, for which a zone size of less than 21 mm was considered resistant. The collected data was entered and analysed using MS-Excel version 11.

RESULTS

During study period of six month, 125 CoNS were identified. Among 125 CoNS isolate 63 were obtained from male and 62 from female patients. The largest proportion of patients (21.6%) fell within the 21-30 years age range, with the 51-60 years group representing the next most frequent category (19.2%) (Graph 1).



Graph 1. Age Wise Distribution Of CoNS



Graph 2. Distribution Of Isolates In Various Clinical Samples

Coagulase-negative staphylococci (CoNS) were most commonly isolated from pus samples (n=66, 52.8%), followed by blood (n=34, 27.2%) and urine (n=19, 15.2%) (Graph 2).

*Staphylococcus hominis* was the most prevalent species (42.4%), succeeded by *Staphylococcus schleiferi* (36.8%), *S. lugdunensis* (15.2%), *S. epidermidis* (4.8%) and *Staphylococcus hemolyticus* (0.8%) (Table 1).

Table 1. Distribution Of Isolated CoNS Species

| Species of CoNS   | Number of CoNS | Percentage% |
|-------------------|----------------|-------------|
| <i>S. hominis</i> | 53             | 42.4        |

|                       |     |      |
|-----------------------|-----|------|
| <i>S. schleiferi</i>  | 46  | 36.8 |
| <i>S. lugdunensis</i> | 19  | 15.2 |
| <i>S. epidermidis</i> | 6   | 4.8  |
| <i>S. hemolyticus</i> | 1   | 0.8  |
| Total                 | 125 |      |

Table 2 shows the distribution of CoNS species in various clinical samples.

Table 2 Species Distribution Of CoNS In Various Clinical Sample

| Sampl es      | <i>S. hominis</i> | <i>S. lugdunensis</i> | <i>S. hemolyticus</i> | <i>S. schleiferi</i> | <i>S. epidermidis</i> | Total number of CONS & % |
|---------------|-------------------|-----------------------|-----------------------|----------------------|-----------------------|--------------------------|
| Pus           | 26                | 13                    | 0                     | 22                   | 05                    | 66 (52.8)                |
| Blood         | 18                | 02                    | 01                    | 12                   | 01                    | 34 (27.2)                |
| Urine         | 05                | 04                    | 0                     | 10                   | 0                     | 19 (15.2)                |
| Ascitic fluid | 01                | 0                     | 0                     | 01                   | 0                     | 02 (1.6)                 |
| Catheter tip  | 03                | 0                     | 0                     | 01                   | 00                    | 04 (3.2)                 |

All 125 isolates were sensitive to both vancomycin and linezolid. Resistance to both penicillin and erythromycin was the most common resistance pattern observed (Table 3).

Table 3. Antibiotic Susceptibility Pattern Of Isolated CoNS Strain

| Antibiotics                           | Sensitive strains |              | Resistant strainsa |              |
|---------------------------------------|-------------------|--------------|--------------------|--------------|
|                                       | Number            | Percentage % | Number             | Percentage % |
| Penicillin                            | 10                | 8            | 115                | 92           |
| Amikacin                              | 115               | 92           | 10                 | 8            |
| Gentamycin                            | 108               | 86.4         | 17                 | 13.6         |
| Erythromycin                          | 24                | 19.2         | 101                | 80.8         |
| Clindamycin                           | 43                | 34.4         | 82                 | 65.6         |
| Ciprofloxacin                         | 89                | 71.2         | 36                 | 28.8         |
| Co-trimoxazole                        | 80                | 64           | 45                 | 36           |
| Cefoxitin                             | 72                | 57.6         | 53                 | 42.4         |
| Vancomycin                            | 125               | 125          | 00                 | 00           |
| Linezolid                             | 125               | 125          | 00                 | 00           |
| Nitrofurantoin (For 19 urine samples) | 15                | 78.94        | 4                  | 26.6         |

Methicillin resistance was detected in 53 (42.4 %) of the 125 isolates, with the remaining 72 (57.6%) being methicillin sensitive. Antibiotic resistance pattern of various isolated CoNS species is detailed in table 4.

Table 4. Antibiotic Resistance Pattern Among Different Species Of CoNS

| Antibiotics                           | <i>S. hominis</i> (n= 53) | <i>S. lugdunensis</i> (n=19) | <i>S. hemolyticus</i> (n=01) | <i>S. schleiferi</i> (n= 46) | <i>S. epidermidis</i> (n=06) |
|---------------------------------------|---------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| Penicillin                            | 49                        | 16                           | 01                           | 45                           | 04                           |
| Amikacin                              | 02                        | 02                           | 00                           | 06                           | 00                           |
| Gentamycin                            | 04                        | 04                           | 00                           | 08                           | 01                           |
| Erythromycin                          | 40                        | 17                           | 01                           | 38                           | 05                           |
| Clindamycin                           | 29                        | 15                           | 01                           | 34                           | 03                           |
| Ciprofloxacin                         | 13                        | 03                           | 00                           | 18                           | 02                           |
| Co-trimoxazole                        | 18                        | 07                           | 01                           | 17                           | 02                           |
| Cefoxitin                             | 21                        | 09                           | 01                           | 19                           | 03                           |
| Vancomycin                            | 00                        | 00                           | 00                           | 00                           | 00                           |
| Linezolid                             | 00                        | 00                           | 00                           | 00                           | 00                           |
| Nitrofurantoin (For 19 urine samples) | 01                        | 00                           | -                            | 03                           | -                            |

DISCUSSION

The significance of coagulase-negative Staphylococci (CoNS) as a cause of hospital-associated infections has grown over past few years. Their increasing resistance to antibiotics

makes the treatment difficult. This study aimed to determine the species distribution and antibiotic susceptibility profiles of CoNS isolated from various clinical samples. In this study, out of 125 CoNS isolates, 63 were from male patients and 62 from female patients, indicating no gender predisposition. Similar findings were reported by Roopa *et al.*<sup>[10]</sup> Conversely, Dilip *et al.*<sup>[11]</sup> reported that CoNS infections were more prevalent in females (54.5%), which authors suggested might be due to higher number of female participants in their study.

In this study, the highest number of CoNS isolates, 27 out of 125 (21.6%), were obtained from patients aged 21-30 years, followed by the 51-60-year age group. Sangwan *et al.*<sup>[12]</sup> also reported a higher prevalence of CoNS infections in the 2<sup>nd</sup> and 3<sup>rd</sup> decades of life. It may be due to post-operative wounds and greater incidence of urinary tract infections in this age group. In the present study majority of CoNS were isolated from pus (66%), followed by blood (27.7%), urine (15.2%) and catheter tips (3.2%). Pus isolates were mainly from post operative wound infections suggesting that CoNS are the significant pathogens causing wound infection. Notably, the highest number of CoNS isolates originated from the surgery and orthopaedics department. The higher isolation of CoNS in patients from these departments may be due to invasive procedures done in these departments.

Our study identified *S. hominis* as the most frequently isolated species (53 isolates, 42.4%), followed by *S. schleiferi* (46 isolates, 36.8%), *S. lugdunensis* (19 isolates, 15.2%), *S. epidermidis* & *S. haemolyticus*. In contrast, Singh *et al.*<sup>[13]</sup> reported *S. epidermidis* (40.7%) as the predominant isolate, followed by *S. haemolyticus* (19.3%) and *S. hominis* (11.9%). Raina *et al.*<sup>[14]</sup> reported the isolation of *S. haemolyticus* (25%) and *S. warneri* (20%) to be the most common CoNS isolates. Similarly, Roopa *et al.*<sup>[10]</sup> reported the isolation of *S. epidermidis* (50.8%) as a predominant pathogen followed by *S. haemolyticus* (26.7%), with other isolated species including *S. lugdunensis* (10.7%), *S. schleiferi* (7.1%) and *S. saprophyticus* (4.46%). This reveals that there exists variation in different species geographically. This variability might be attributed to differing antimicrobial pressures in diverse hospital environments, which could influence the CoNS colonization of skin and mucous membranes, explaining the above findings.

Antibiotic susceptibility testing in our study revealed the highest resistance to penicillin (92%) and erythromycin (80.8%). Notably, all CoNS isolates demonstrated 100% sensitivity to both vancomycin and linezolid. Such a high degree of resistance to penicillin (84.5%), was reported by Singh *et al.*,<sup>[13]</sup> while Dilip *et al.*<sup>[11]</sup> reported 95.5% of their isolates resistant to penicillin and 80.9% resistant to erythromycin. Bora *et al.*<sup>[15]</sup> also found substantial resistance to penicillin (96.5%), as well as to ciprofloxacin (57.1%) and oxacillin (45.5%), and consistent with our findings, they also reported 100% sensitivity to linezolid and vancomycin among their isolates.

In our study, 42.4 % of the 125 isolates were identified as methicillin resistant CoNS (MRCoNS). Roopa *et al.*<sup>[10]</sup> reported MRCoNS prevalence of 33%. She also reported resistance to multiple antibiotics, including ampicillin and cotrimoxazole by MRCoNS. Whereas Singh *et al.*<sup>[6]</sup> reported higher MRCoNS of 57.6%. The high occurrence of methicillin resistance among CoNS could be attributed to the exchange of resistance genes between CoNS and *S. aureus*. In the recent years there is increase frequency of drug resistant strains of CoNS, therefore early detection of MRCoNS and reduction in indiscriminate use of antibiotics will be the necessary approach to reduce the emergence of drug resistant strains.

## CONCLUSION

CoNS are the major contributors to the hospital associated infections, *Staphylococcus hominis* was the most common

species identified. Resistance to commonly used drugs like penicillin and erythromycin is prevalent among CoNS. Therefore, prompt identification, speciation and antibiotic susceptibility profiling of CoNS are crucial for effective patient management in light of these emerging resistance patterns.

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